Dietary Intake of Phytate, Zinc and Calcium of Self-Selected Diets of Ubon Ratchathani and Bangkok Subjects, Thailand

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Abstract

The dietaty intakes of phytate, zinc and calcium of volunteers in Ubon Ratchathani (northeast Thailand) and Bangkok metropolitan were studied. The selected subjects were 10 men and 10 women from each area. The daily diets were collected for 5 consecutive days using duplicate portion technique. The diets were blended, aliquoted, mixed, freeze-dried and homogenized. Phytate was analysed by using ion exchange coupled with colorimetric method. Zinc and calcium were analysed by atomic absorption spectrophotometer. The total intakes of phytate were 1104.8 $\pm$ 965.2 and 1139.3 $\pm$ 481.1 mg/day for males and females in Ubon Ratchathani, 1304.7 $\pm$ 956.2 and 997.1 $\pm$ 435.1 mg/day for males and females in Bangkok. The zinc intakes were 20.0 $\pm$ 8.8, 12.7 $\pm$ 4.6, 7.7 $\pm$ 1.8 and 6.1 $\pm$ 1.2 mg/day for males and females in Ubon Ratchathani and for males and females in Bongkok, respectively. The calcium intakes were 524.6 $\pm$ 259.9, 379.9 $\pm$ 111.4 for males and females in Ubon Ratchathani and 366.5 $\pm$ 150.5, 286.7 $\pm$ 68.7 mg/day for males and females in Bangkok. The calculated phytate/zinc molar ratios were 7.5, 3.9 for males and females in Ubon Ratchathani and 16.2 and 17.5 for males and females in Bangkok This study indicated that subjects from Bangkok have a phytate/zinc ratio higher than those in Ubon Ratchathani and higher than 12. This may effect the availability of some micronutrients such as zinc, calcium and iron. The daily intakes of zinc and calcuim in these two groups were low compared to Thai RDA.

Key word : Phytate, Zinc, Calcium, Diet, Thailand

Zinc is an essential trace element for human beings. Clinical manifestations of dietary deficiency are retarded growth, depressed immune function, anorexia, dermatitis, altered reproductive performance, diarrhea and alopecia(1). Phytate (myo-inositol hexa kis dihydrogen phosphate) is widely distributed in foods of plant origin. Animal studies, *in vivo*, have shown that dietary phytate

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significantly reduces zinc absorption by the formation of insoluble complexes (Davies & Nightingale, 1975; Graf & Eaton, 1984)(2,3). Moreover, the presence of calcium has been found to have an enhancing effect on precipitation of the phytate zinc complex (Cheryan, 1980)(4).

The prevalence of trace element deficiencies is predicted to be higher in populations subsisting on cereals as the staple food because cereals are high in minerals and also high in phytate. Use of the phytate/Zn molar ratio was a good indicator of the bioavailability of dietary zinc in rats(6-8). In 1983, Cossak and Prasad(9) used phytate x calcium/Zn millimolar ratios, their calculations suggested that ratios equal to or greater than 200 might induce marginal zinc deficiency. This millimolar ratio was also used by Davies et al in 1985(10) and Brinda et al in 1986(11). Thailand is a developing country, the population of which consumes mainly cereals (rice and sticky rice). Phytate contents of rice, sticky rice and other food items were studied earlier(12). It was shown that some food items were high in phytate such as sesame seeds and soy bean. The contents in rice were shown to be considerably lower. Therefore, it is interesting to study the dietary intake of phytate, zinc and calcium and using these molar ratios as indicaters. We have studied phytate/Zn molar ratio in school children in a North East school in Thailand and found that the ratios were 4.5 and 2.8 for normal and PEM respectively(13). In the present study, an attempt was made to estimate phytate, zinc and calcium intakes of selected volunteer subjects living in Ubon Ratchathani province (northeast Thailand-rural area) and Bangkok metropolitan (urban area), and to calculate phytate/Zn molar ratio and phytate x calcium/Zn millimolar ratio and to compare the two areas.

MATERIAL AND METHOD

Collection of samples

The total daily diets were collected from twenty volunteer subjects who were active working persons (10 males, 10 females) in a rural area, Ubon Ratchathani province and in the urban area of Bangkok. These subjects were selected based on their willingness to participate. Food samples were collected by the duplicate portion technique. The subjects were requested to collect exact duplicate portions of all their food and beverages (including water) for a period of 5 consecutive days. The samples were kept in a clean plastic container and stored in the refrigerator (4°C) before being transported to the laboratory under frozen conditions.

Sample preparation

The samples of each day were weighed and blended using a Robot blender with a plastic bowl and titanium blade. The collected fluid of the same day was added to complete the sample homogenization. The proportional aliquots of homogenates from 5 days were combined, mixed and freeze-dried with HETOSICC lyophilizer. Then the freeze-dried samples were homogenized again. The moisture contents of all samples were determined before and after freeze-drying process.

Phytate determination

Dry, powdered samples were analyzed for phytate using the improved Ion Exchanged Method of Ellis and Morris, 1983(14). Sodium phytate (No-P5756) from Sigma Chemical Company was used as standard.

Mineral analysis

The minerals, zinc and calcium were determined. A 1-2 g of dried sample was weighed in a porcelain crucible which had previously been ignited and cooled. The crucible and content was heated on a hot plate until charred at 100°C. It was placed in a muffle furnace at the initial temperature of 100°C. The temperature was increased to 450° at 50°C/h and was heated overnight. The crucible was removed from the furnace and cooled to room temperature. The ash was moistened with 1-3 ml water, and evaporated on a hot plate. The crucible was returned to the furnace at 200°C and the temperature was raised stepwise to 450°C, and held for 1-2 h. This was repeated until the sample was completely combusted. Residue was dissolved in exact volume (10.0-30.0 ml) of 0.1 M HNO_3 according to the concentration of metal to be determined(15). Zinc and calcium was determined by Atomic Absorption Spectrophotometer (Perkin Elmer, 1100B).

RESULT

The age, weight and height of subjects are shown in Table 1. The dietary intakes of phytate, zinc, and calcium according to sex are shown in Table 2. The zinc intakes of males were significantly higher than that of females ($P<0.05$) in Ubon Ratchathani. Calcium and zinc intakes were significantly ($P<0.05$) higher in males than in females

Table 1. Ages, weights and heights of Ubon Ratchathani and Bangkok subjects

	Ubon Ratchathani		Bangkok	
	Male	Female	Male	Female
Age, yrs	32.5 ± 4.9	27.5 ± 2.3	29.6 ± 5.2	29.9 ± 5.9
Weight, kg	54.7 ± 7.5	49.9 ± 4.5	57.7 ± 6.7	50.3 ± 4.4
Height, cm	162.6 ± 4.9	150.0 ± 3.9	166.5 ± 6.0	157.4 ± 5.6

Table 2. Daily dietary intake of phytate, zinc and calcium of Ubon Ratchathani and Bangkok subjects (Mean±SD and min.-max.)

Area	Phytate, mg	Zinc, mg	Calcium, mg
Ubon Ratchathani Male	1,104.8 ± 965.2 (209.0 - 3,076.0) n=9	20.0 ± 8.8 (11.0 - 38.2) n=10	524.6 ± 259.9 (242.2 - 910.1) n =10
Female	1,139.3 ± 481.1 (528.0 - 1,946.0) n=7	12.7 ± 4.6* (7.8 - 23.4) n=10	379.9 ± 111.4 (228.2 - 562.7) n=10
Total	1119.8 ± 767.9 (209.0 - 3,076.0) n=16	16.4 ± 7.8 (7.8 - 38.2) n=20	452.3 ± 208.3 (228.2 - 910.1) n=20
Bangkok Male	1,304.7 ± 956.2 (228.0 - 2,817.0) n=10	7.7 ± 1.8[a] (5.3 - 11.5) n=10	366.5 ± 150.5 (74.8 - 600.9) n=10
Female	997.1 ± 435.1 (327.0 - 1,395.0) n=7	6.1 ± 1.2*[b] (4.7 - 8.2) n=10	286.7 ± 68.7[b] (205.1 - 380.1) n=10
Total	1,178.1 ± 780.8 (228.0 - 2,817.0) n=17	6.9 ± 1.7[c] (4.7 - 11.5) n=20	336.6 ± 106.5[c] (205.2 - 600.9) n=20

* significant difference between sex (P<0.05)
a significant difference between province in male (P<0.05)
b significant difference between province in female (P<0.05)
c significant difference between province in both sex (P<0.05)

of Bangkok. When comparing Ubon Ratchathani and Bangkok, it was found that zinc was significantly (P<0.01) higher in males of Ubon Ratchathani than in males of Bangkok. Zinc daily intakes were found to be significantly higher in males than in females of both areas (P<0.05). When comparing the two areas, daily intakes of zinc in males and females of Ubon Ratchathani were significantly higher than those of Bangkok (P<0.05). While calcium daily intake was higher in females of Ubon Ratchathani than that of females in Bangkok (P<0.05). From all the subjects, it was found that both zinc and calcium daily intakes were significantly higher in Ubon Ratchathani than in Bangkok. The zinc and calcium daily intakes of Ubon Ratchathani subjects were 16.4 mg and 452.3 mg, respectively. These were 109 per cent and 56.5 per cent of Thai RDA (15 mg and 800 mg). For the Bangkok area, zinc and calcium daily intakes were 6.9 and 336.6 mg which are 46 and 42 per cent of Thai RDA.

Phytate/Zn molar ratios and phytate x Ca/Zn millimolar ratios are presented in Table 3 and Fig. 1. Phytate/Zn molar ratios were significantly higher ($P<0.05$) in males of Bangkok than males of Ubon Ratchathani. Also the ratios were higher ($P<0.05$) in females of Bangkok than of Ubon Ratchathani. The same result was observed in all the subjects. Phytate x Ca/Zn millimolar ratios were significantly higher ($P<0.05$) in males and all the subjects from Bangkok than in those of Ubon Ratchathani. These indexes have been used to indicate the effect of phytate on bioavailability of zinc in human. In rats, it has been shown that if phytate/Zn ratio in the diet is less than 12, phytate will have no effect on zinc bioavailability(16). While in humans the ratio of 15 has been shown to associate with reduced zinc bioavailability(17,18), whereas others have used the critical ratio of 20(11,18-20).

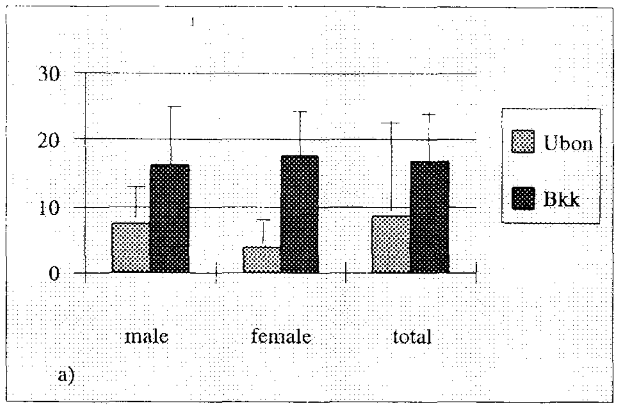
DISCUSSION

It has been indicated from some studies that requirements of some minerals such as calcium may have ethnic differences(22,23). There was wide variation in daily intakes of calcium from 441 mg/day to 734 mg/day. In Thailand, the intake of calcium was found to be 456 mg/day(24). It is comparable to this study, 452 mg/day for Ubon Ratchathani and 337 mg/day for Bangkok. However, there are extensive studies on calcium status and bone mass in the Thai population. Zinc intake in healthy adults was reported to be 6.3 mg/day and 5.5 mg/day for men and women in Bangkok(25) which is also

Table 3. Phytate/Zn molar ratio and phytate x Ca/Zn millimolar ratio of Ubon Ratchathani and Bangkok subjects (Mean±SD and min.-max.)

Area	Phytate/Zn	Phytate x Ca/Zn
Ubon Ratchathani		
Male	7.5 ± 5.9 (1.6 - 17.5) n=9	63.7 ±52.4 (10.2 - 154.4) n=9
Female	3.9 ± 3.6 (6.1 - 15.5) n=7	92.9 ± 37.2 (53.2 - 148.1) n=7
Total	8.5 ±16.7 (1.6 - 17.5) n=16	76.5 ± 47.4 (10.2 - 154.4) n=16
Bangkok		
Male	16.2 ± 10.7[a] (3.8 - 32.6) n=10	173.0 ± 138.0[a] (24.5 - 434.6) n=10
Female	17.5 ±7.8[b] (4.6 - 27.8) n=7	122.0 ± 61.9 (33.6 - 182.9) n=8
Total	16.7 ± 9.4[c] (3.8 - 32.6) n=17	152.0 ± 113.3[c] (24.5 - 434.6) n=17

a significant difference between province in male ($P<0.05$)
b significant difference between province in female ($P<0.05$)
c significant difference between province in both sex ($p<0.05$)



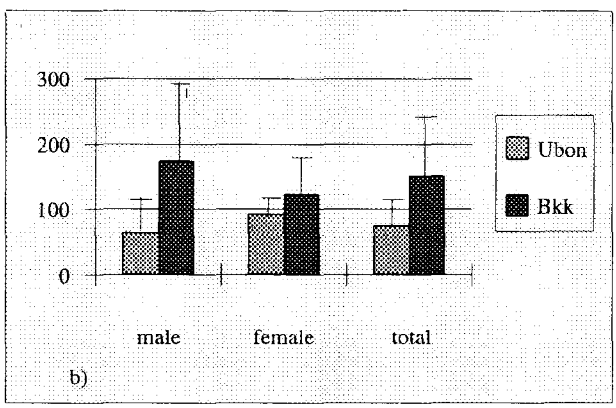


Fig. 1. a) Phytate/Zn molar ratio and b) phytate x Ca/Zn millimolar ratio of Ubon Ratchathani (Ubon) and Bangkok (Bkk) subjects.

comparable to this study, 7.7 mg/day and 6.1 mg/day for males and females in Bangkok, respectively.

For phytate, the daily intake in this study, was found to be low compared to some previous studies(26). It was reported that phytate was mostly rich in some oilseed, such as sesame seed, soybean(12).

Though subjects in Ubon Ratchathani consumed more rice and/or sticky rice than those in Bangkok, the phytate/zinc molar ratio of Bangkok subjects was significantly higher than those in Ubon Ratchathani. This can be speculated from the detail of food items eaten, that subjects in Bangkok eat more varieties of foods. Some of them are made of materials that are high in phytate. These are snack food and desserts made from mung bean, peanut and some of the subjects ate soybean curd which is also expected to contain phytate in a higher level. While most of the subjects in Ubon Ratchathani ate only main meals. These meals were composed of meat, fish, eggs, fruit and vegetables. The staple foods, rice and sticky rice, are quite low in phytate. Therefore, it does not contribute much to the total daily intake, even although it was consumed more by the Ubon Ratchathani group. This slightly high in phytate intake together with a significantly lower zinc intake make the higher ratio in Bangkok group. However, only males in Bangkok had a ratio more than 15 (16.2), but still not more than 20. Recently, we reported the mean phytate/zinc molar ratios of school children of 4.5 and 2.8(13) which is similar to Ontario preschool children of 4.1(20). Cossak and Prasad suggested that millimolar ratios of phytate x Ca/Zn equal to or greater than 200 might induce marginal zinc deficiency(9). In this study, the group that had a ratio close to 200 was males in Bangkok with a mean ratio of 173 (Table 3). They may be at risk of zinc unavailability from food intake. The phytate /Zn ratio was also in the high trend in this same group. Anyhow, this high trend still was not up to the critical ratio. The phytate x Ca/Zn millimolar ratio of same group was also in high trend but was not as high when compared to those of American vegetarians (women with 91-434 and men with 45-548). Asian Indian vegetarians were 127-315 for women and 123-405 for men, while female Nepalese vegetarians were 54-533(26).

SUMMARY

Subjects in Ubon Ratchathani consumed more rice and/or sticky rice than those in Bangkok but the phytate intakes were slightly lower, while zinc intakes were significantly higher. It may be speculated that subjects in Bangkok ate more snacks and desserts made of legumes and seeds (such as soybean, sesame seeds) which are in the high phytate groups of food. This could be the result of higher phytate/zinc and phytate x calcium/zinc ratios of subjects in Bangkok than those in Ubon Ratchathani. However, the ratios were still below the critical points reported to effect zinc bioavailability.

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REFERENCES

1. Hambidge KM, Casey CE, Krebs NF. Zinc. In: Merth w, eds. Trace Elements in Human and Animal Nutrition, vol.2. 5th ed. Orlando: Academic Press, 1986:1-137.
2. Davies NT, Nightingale R. The effect of phytate on intestinal absorption and secretion of zinc and whole body retention of zinc, copper, iron and manganese in rats. Br J Nutr 1975;34:243-58.
3. Graf E, Eaton JW. Effects of phytate on mineral bioavailability in mice. J Nutr 1984;114:1092-198.
4. Cheryan M. Phytic acid interactions in food systems. CRC Crit Rev Food Sci Nutr 1980;13: 297-335.
5. Oberleas D, Prasad AS. Zinc and copper. In Prasad AS. ed. Trace Elements in Human Health and Disease. vol.1. Newyork: Academic Press, 1976: 155-62.
6. Davies NT, Olpin SE. Studies on the phytate/zinc contents in diets as a determinant of Zn availability to young rats. Br J Nutr 1979; 41:591-603.
7. Morris ER, Ellis R. Effect of dietary phytate/zinc molar ratio on growth and bone zinc response of rats fed semipurified diets. J Nutr 1980; 110: 1037-45.
8. Lo GS, Settle SL, Steinke FH, et al. Effect of phytate/zinc molar ratio and isolated soybean protein on zinc bioavailavility. J Nutr 1981;111:2223-35.
9. Cossack ZT, Prasad AS. Effect of protein source on the bioavailabitily of zinc in human subjects. Nutr Res 1983;3:23-31.
10. Davies NT, Carswell AJP, Mills CF. The effect of variation in dietary calcium intake on the phytate-zinc interaction in rats. In : Mills CF, Bremner I, and Chester JK, eds. Trace Element in Man and Animals. Proceeding of the Fifth International Symposium on Trace Elements in Man and Animals, 1985:456-7.
11. Bindra GS, Gibson RS, Thompson L. (Phytate) (calcium)/(zinc) molar ratios in Asian immigrant lacto-ovo vegetarian diets and their relationship to zinc nutriture. Nutr Res 1986;6:475-83.
12. Nititham S, Srianujata S. Phytate levels in selected Thai foods. J Nutr Assoc Thailand 1991; 25:78-85.
13. Nititham S, Srianujata S. Phytate/zinc molar ratio and daily dietary intakes of some minerals in school children in a school of lower Norteast Thailand. Abstract of The seventh Asian Congress of Nutriton, Beijing, China. October 7-11, 1995: 542.
14. Ellis R, Morris ER. Improved Ion-Exchange Phytate Method. Cereal Chemistry 1983;60:121-4.
15. Jorhem L. Determination of metals in foodstuffs by atomic absorption Spectrophotometer after dry ashing: NMKL Interlaboratory Study of Lead, Cadmium, Zinc, Copper, Iron, Chromium, and Nickel. J AOAC Inter 1993:798-813.
16. Morris ER, Ellis R. Bioavailability to rats of iron and zinc in wheat bran:Response to low phytate bran and effect of the phytate/zinc molar ratio. J Nutr 1980;110:200-2010.
17. Turnlund JR, King KC, Keyes WR, et al. A stable isotope study of zinc absorption in young men: effects of phytate and a-cellulose. Am J Clin Nutr 1984;40:1071-7.
18. Navert B, Sandstrom B. Reduction of the phytate content of bran by leavening in breads and its effect on zinc absorption in man. Br J Nur 1985; 52: 47-53.
19. Oberleas D, Harland BF, Phytate content of foods: effect on dietary zinc availability. J Am Diet Assoc 1981;79:433-6.
20. Gibson RS, Smitr Vanderkooy PD, Thompson L. Dietary phytate x calcium/zinc mM ratios and zinc nutriture in some Ontario preschool children. Biol Trace Elem Res 1991;30:87-94.
21. Eton PM, Wharton PA, Wharton BA. Nutrient intake of pregnant Asian woman at Sorrento Maternity Hospital, Birmingham. Br J Nutr 1984; 52: 457-68.
22. Lee WT. Requirements of calcium: are there ethnic differences? Asia Pacific. J Clin Nutr 1993; 2:183-90.
23. Kim KK, Yu ES, Liu WT, et al. Nutritional status of Chinese-, Korean-, and Japanese- Ameican elderly. J Am Dietetic Assoc 1993;63:1416-22.
24. Chitchumroonchokchai C, Kongkachuichai R, Sirichakwal P, et al. Macro element intake of Thai adult subjects: chemical analyses of diets using the duplicate portion sampling technique. Rama Med J 1993;16:168-73.
25. Songchitsomboon S, Komindr S, Piasea N. Zinc and copper intake and sources in healthy adults living in Bangkok and surrounding districts. Biol Trace Elem Res 1998;61:97-104.
26. Ellis R, Kelsay JL, Reynolds RD, et al. Phytate/zinc and phytate x cacium/zinc mM ratios in self-selected diets of Americans, Asian Indians, and Nepalese. J Am Diet Assoc 1987;87:1043-7.

การศึกษาการได้รับไฟเตต สังกะสีและแคลเซียม จากอาหารปกติของอาสาสมัครที่จังหวัดอุบลราชธานี และกรุงเทพมหานคร†

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ได้ศึกษาการได้รับไฟเตตสังกะสีและเคลเซียมจากอาหารของอาสาสมัคร ที่จังหวัดอุบลราชธานีและกรุงเทพฯ โดยคัดเลือกอาสาสมัครชาย 10 คนและหญิง 10 คนจากแต่ละจังหวัด ทำการเก็บอาหารด้วยวิธี duplicate portion เป็นเวลา 5 วันติดต่อกัน นำตัวอย่างมาปั่นให้เป็นเนื้อเดียวกัน และทำให้แห้งด้วยวิธี Freeze-dry ตัวอย่างอาหารที่ได้นำไปวิเคราะห์หาไฟเตตด้วยวิธี ion exchange ส่วนสังกะสีและแคลเซียมวิเคราะห์ด้วย Atomic Absorption Spectrophotometer ปริมาณไฟเตตที่ได้รับจากอาหารของอาสาสมัครชายและหญิงมีค่าเฉลี่ยเท่ากับ 1104.8 ± 965.2 และ 1139.3 ± 481.1 มก.ต่อวันสำหรับอุบลราชธานี และ 1304.7 ± 956.2, 997.1 ± 435.1 มก.ต่อวัน สำหรับกรุงเทพฯ การได้รับสังกะสีจากอาหารมีค่าเฉลี่ยเท่ากับ 20.0 ± 8.8, 12.7 ± 4.6, 7.7 ± 1.8 และ 6.1 ± 1.2 มก.ต่อวัน สำหรับอาสาสมัครชายและหญิงจากอุบลราชธานีและกรุงเทพฯ ตามลำดับ ส่วนการได้รับแคลเซียมจากอาหารมีค่าเฉลี่ยเท่ากับ 524.6 ± 259.9, 379.9 ± 111.4, 366.5 ± 150.5 และ 286.7 ± 68.7 มก.ต่อวัน สำหรับอาสาสมัครชายและหญิงจากอุบลราชธานีและกรุงเทพฯ ตามลำดับ การศึกษาครั้งนี้ชี้ให้เห็นว่า อาสาสมัครจากกรุงเทพฯ มีอัตราไฟเตตต่อสังกะสีสูงกว่าอาสาสมัครจากอุบลราชธานี และมีค่าเกิน 12 ซึ่งอาจส่งผลถึงการนำแร่ธาตุปริมาณน้อยไปใช้ในร่างกาย นอกจากนี้ยังพบว่า ปริมาณสังกะสีและแคลเซียมที่ได้รับจากอาหารของอาสาสมัครทั้งสองกลุ่มมีค่าต่ำกว่าข้อกำหนดสารอาหารที่ควรได้รับประจำวันสำหรับคนไทย (Thai RDA).

คำสำคัญ : ไฟเตต, สังกะสี, แคลเซียม, อาหาร, ไทย

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